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Application of a Multiplex Polymerase Chain Reaction Assay for the Simultaneous Confirmation of *Listeria monocytogenes* and Other *Listeria* Species in Turkey Sample Surveillance†

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ABSTRACT

A multiplex polymerase chain reaction was developed to simultaneously identify *Listeria monocytogenes* and species of the genus *Listeria*. Two sets of primers were used, with the first amplifying a 938-bp region of the 16S rRNA gene that is highly conserved in all *Listeria* species and the second amplifying a 174-bp region of the listeriolysin (*hlyA*) gene of *L. monocytogenes*. Thus, isolates of *Listeria* spp. yield a single 938-bp product, whereas *L. monocytogenes* isolates yield both the 938-bp product and a 174-bp product. The specificity of the assay was verified with all six *Listeria* species and 11 serotypes of *L. monocytogenes,* as well as nonrelated bacteria. The multiplex PCR assay was used to determine the incidence of *Listeria* spp., especially *L. monocytogenes,* in mechanically separated turkey samples ($n = 150$ samples). *L. monocytogenes* strains were selected by using the University of Vermont two-step enrichment protocol and plating to selective Palcam agar. The multiplex PCR assay was used for verification of presumptive *Listeria* colonies. Approximately 38% of mechanically separated turkey samples (57 of 150) yielded *L. monocytogenes*; an additional 18% of these samples (27 of 150) harbored other *Listeria* spp. Fifty-one percent (29 of 57) of the *L. monocytogenes* isolates were of serogroup 1, 44% (25 of 57) were of serogroup 4, and 2% (1 of 57) were assigned to serogroups other than 1 and 4.

Listeria monocytogenes is a major bacterial foodborne pathogen that accounts for an estimated 2,500 cases of human illness (meningitis, encephalitis, sepsis, fetal death, prematurity) and 504 deaths annually, resulting in an estimated loss of $200,000,000 *(9).* Nearly 90% of all reported cases result in hospitalization. The mortality rate for clinical listeriosis (~25%) is higher than that for any other foodborne illness *(26).* The U.S. Public Health Service has set the goal of reducing human bacterial foodborne illness from the 1997 baseline by the year 2010. The rate of listeriosis cases in the United States is projected to decline by ~50% from the 1997 baseline (0.5 cases per 100,000 people) by 2010 (0.25 cases per 100,000 people).

L. monocytogenes has been implicated in major foodborne epidemics *(29)* involving the consumption of contaminated pâté *(25),* pork tongues *(19),* soft cheeses *(23),* and coleslaw *(31).* The most recent multistate outbreak of human listeriosis in the United States, ascribed to serotype 4b (101 cases, resulting in 22 deaths), was linked to delicatessen meats, including turkey *(2).*

Sporadic human cases of listeriosis have also been epidemiologically linked to the consumption of undercooked poultry products *(32).* Analysis of risk factors associated with infections in the United States has indicated that cancer patients and immunocompromised individuals, in whom 69% of listeriosis cases occur, were more likely than control subjects to have eaten undercooked poultry (odds ratio = 3.3) *(34).* In 1989, contaminated turkey franks were linked to a listeriosis fatality for a breast cancer patient in Oklahoma *(38).* After isolating *L. monocytogenes* 1/2a of the same isoenzyme type from the patient and from the turkey frankfurters, the U.S. Department of Agriculture Food Safety and Inspection Service requested a class 1 (voluntary) recall of 600,000 lb of the product *(1).*

L. monocytogenes is present at low levels in live market weight poultry, including turkeys. However, during the U.S. Department of Agriculture Food Safety and Inspection Service Nationwide Young Turkey Microbiological Baseline Data Collection Program, *L. monocytogenes* was found in 5.9% of turkey carcass rinses and in 30.5% of ground turkey meat samples examined *(36).* The increased *L. monocytogenes* recovery for the ground product may reflect contamination during processing. In another report, although *L. monocytogenes* was not cultured from the intestinal tracts and ceca of turkeys at slaughter, it was recovered from processing water, from mechanically deboned meats, and from the hands and gloves of 34% of the meat cutters *(15).* Additional studies involving DNA profiles of *L. monocytogenes* indicate that significant cross contamination occurs during poultry processing *(27).*

The polymerase chain reaction (PCR) is a rapid, specific, and sensitive adjunct to standard methods for the confirmation of *Listeria (3, 4, 22).* PCR-based strategies for

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the identification of *L. monocytogenes* have targeted a number of sequences, including the 16S rRNA *(5), iap (6, 8), ImA (21, 35),* and *hlyA (7, 13, 17)* genes. Multiplex formats for the 16S rDNA gene and the *iap* gene, which encodes the p60 protein for all *Listeria* species, as well as the *hlyA* sequences, which code for the unique listeriolysin virulence factor of *L. monocytogenes,* have been detailed *(5).*

Genetic typing has been used to characterize and demonstrate the possible relationship between clinical and food isolates of *L. monocytogenes (11, 14, 16).* PCR-based amplification of enterobacterial repetitive intergenic consensus (ERIC) sequences has been used to profile field strains and elucidate the epidemiology of human pathogens, including *L. monocytogenes (33).* We previously reported that the diversity of ERIC patterns generated for *Arocbacter butzleri* strains recovered from mechanically separated turkey meat indicated multiple sources of contamination *(24).*

In 1999, nearly 48% of all meat-associated recalls in the United States were linked to *L. monocytogenes (37).* Because of the imposed zero tolerance for ready-to-eat products in the United States and the subsequent costs involved in product recalls, the isolation and correct identification of *L. monocytogenes* and its clear differentiation from *Listeria innocua* and other closely related species are crucial. Herein, we describe a multiplex PCR assay that amplifies the highly conserved 16S rRNA gene of *Listeria* spp. *(5)* as well as the *hlyA* gene of *L. monocytogenes (12).* As a result, the assay concurrently identifies members of the genus *Listeria* and *L. monocytogenes.* The optimized assay was then used to confirm the recovery of *L. monocytogenes* from mechanically separated turkey meat.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. Bacterial strains were obtained from the National Animal Disease Center Culture Collection (Table 1). Bacteria were maintained at 4°C on Trypticase soy agar slants with 0.6% yeast extract (TSA-YE) and were subcultured to TSA-YE plates overnight at 37°C prior to colony PCR.

PCR primers. For initial optimization, DNA was extracted with cesium chloride gradients and quantitated as previously described *(41).* Two primer sets were used. Set I consisted of primers Lis-1 and Lis-2, and set II consisted of primers U1 and LI1. Primers Lis-1 (5′-GCA-TCT-GCA-TTC-AAT-AAA-GA-3′) and Lis-2 (5′-TGT-CAC-TGC-ATC-TCC-GTG-GT-3′) amplify a 174-bp region of the *hlyA* gene in *L. monocytogenes (12).* Primers U1 (5′-CAG-CMG-CCG-CGG-TAA-TWC-3′, where M denotes A or C, and W denotes A or T) and LI1 (5′-CTC-CAT-AAA-GGT-GAC-CCT-3′) target a 938-bp 16S rRNA sequence in members of the genus *Listeria (8).* Primers were commercially synthesized (Integrated DNA Technologies, Inc., Coralville, Iowa).

PCR amplification. Amplification was performed with a volume of 50 μl containing 0.5 ng of sample DNA, 50 pmol each of Lis-1, Lis-2, UI, and LI1, 1.25 U of *Taq* DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), 200 μM (each) dATP, dCTP, dTTP, and dGTP, 10 mM Tris-HCl, 50 mM KCl, and 6.5 mM $MgCl_2$. The reaction mixture was overlaid with mineral oil, and the tubes were placed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.). The samples were subjected to an initial denaturation step of 94°C for 4 min, followed by 25 amplification cycles of 1 min at 94°C (denaturation), 1 min at 60°C (primer annealing), and 1 min at 72°C (primer extension). A primer extension step of 72°C for 5 min followed the final amplification cycle. PCR reaction products were separated on 1.5% agarose gels (60 V for 1.5 h) in a horizontal gel bed (8.3 by 6.0 cm) with Tris-borate-EDTA as the running buffer. DNA molecular weight marker VI (Boehringer Mannheim) was included for base pair size comparison. The gel was then stained with ethidium bromide and visualized according to Sambrook et al. *(30).*

TABLE 1. *Specific amplification of ATCC reference strains with primers targeted to the genes encoding 16S rRNA of* Listeria *spp. (938-bp product) and the listeriolysin (*hlyA*) gene of* L. monocytogenes *(938- and 174-bp products)*

Bacteria	938-bp PCR product	938- and 174-bp PCR product
Listeria monocytogenes ATCC 19111	+	+
L. monocytogenes ATCC 19112	+	+
L. monocytogenes ATCC 19113	+	+
L. monocytogenes ATCC 19114	+	+
L. monocytogenes ATCC 13932	+	+
Listeria grayi ATCC 19120	+	−
Listeria innocua ATCC 33090	+	−
Listeria ivanovii ATCC 19119	+	−
Listeria murrayi ATCC 25401	+	−
Listeria welshimeri ATCC 35897	+	−
Campylobacter jejuni	−	−
Escherichia coli O157:H7	−	−
Erysipelothrix rhusiopathiae	−	−
Enterobacter cloaceae	−	−
Hafnia alvei	−	−
Klebsiella pneumoniae	−	−
Proteus vulgaris	−	−
Pseudomonas aeruginosa	−	−
Rahnella aquatilis	−	−
Salmonella Typhimurium	−	−
Streptococcus suis	−	−
Staphylococcus aureus	−	−
Yersinia enterocolitica	−	−
Yersinia aldovae	−	−
Yersinia ruckeri	−	−

For colony PCR, purified colonies or a loop of bacterial growth from a plate was resuspended in 150 μl of sterile distilled water in a microcentrifuge tube. The sample was incubated in a boiling water bath for 15 min, pelleted for 30 s (microcentrifuge Model 235C, Fisher Scientific), and immediately placed on ice. An aliquot of the supernate (5 to 10 μl) was used as the DNA template and amplified as described above for purified DNA.

***Listeria* isolation.** Mechanically separated turkey meat samples (n = 150) were obtained from a single Midwestern turkey establishment on four occasions. The first (1 July 1997) and second (14 July 1997) trials involved 25 samples each. Trials 3 (10 September 1997) and 4 (10 December 1997) involved 50 samples each. The samples were collected by plant personnel and shipped overnight on ice to the National Animal Disease Center, Ames, Iowa. Once the samples were received, 25 g of each sample was placed in 225 ml of UVM I broth and incubated at 30°C for 24 h. After incubation, 0.2 ml of the UVM I enrichment was trans-

ferred into 10 ml of UVM II (30°C for 24 h). A loopful of growth from UVM II was subcultured onto Palcam selective agar (Oxoid, Orangeburg, N.Y.) and incubated microaerobically (5% O_2, 10% CO_2, and 85% N_2). Suspect *Listeria* colonies, black because of esculin hydrolysis, were transferred to TSA-YE slants, incubated at 37°C for 24 h, and stored at 4°C for final PCR confirmation and serotyping.

PCR template preparation. Typical *Listeria*-like colonies (black colonies) were collected from the surfaces of Palcam plates with a bacterial loop and suspended in 250 μl of sterile distilled water, boiled for 15 min, and centrifuged at 10,000 rpm for 1 min. An aliquot of the supernate (5 to 10 μl) was used as the DNA template and amplified as described above for purified DNA.

Serological identification of *L. monocytogenes* from mechanically separated turkey meat. Presumptive *Listeria* spp. isolates ($n = 66$) were transferred to TSA-YE and refrigerated for serotyping. *L. monocytogenes* strains were reconfirmed by PCR and grouped by using the rapid slide test for serogroups 1 and 4 (Difco Laboratories, Detroit, Mich.) according to the manufacturer's instructions. After mixing for 1 to 2 min, the slide was read for agglutination. If agglutination was observed, a sample was considered positive. If no clumping was seen, a sample was considered negative.

PCR-based ERIC analysis. ERIC profiles were obtained for all isolates from mechanically separated turkey meat that were confirmed to be of serotype 1 ($n = 29$). ERIC profiles were compared with those generated by *L. monocytogenes* strains (NADC 2664 to NADC 2678, $n = 15$) that had been recovered from turkey franks associated with a listeriosis fatality *(38).* A total of 44 *L. monocytogenes* strains were grown on TSA-YE plates, and DNA was extracted with the High Pure PCR Template Preparation Kit (Boehringer Mannhiem) according to the manufacturer's instructions. Genomic DNA was quantified spectrophotometrically at emission wavelengths of 260 and 280 nm (Beckman DU-600).

All reaction volumes (50 μl) contained four dNTPs at 0.2 mM each, 1.25 U of *Taq* DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 25 pmol each primer, and 50 ng of template (genomic) DNA. Reactions were overlaid with mineral oil. Primers for the ERIC PCR (ERIC1R [5′-ATGTAACGTCCTGGGGATTCA C-3′] and ERIC2 [5′-AAGTAAGTGACTGGGGTGAGCG-3′]) were used as described *(18, 23, 39).* Amplification conditions included an initial denaturation step of 94°C for 5 min, followed by 40 cycles of consecutive denaturation for 1 min at 94°C, annealing for 1 min at 25°C, and primer extension for 4 min at 74°C, followed by a final extension for 10 min at 74°C. Amplification was performed with a DNA Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.). PCR products were electrophoretically separated (120 V for 45 min) by using 1.5% agarose gel and Tris-borate-EDTA as the running buffer. The gels were stained with ethidium bromide, rinsed, visualized, and photographed on a Gel Doc (Bio-Rad Laboratories, Richmond, Calif.). Resulting patterns were compared visually.

RESULTS

PCR specificity testing. American Type Culture Collection (ATCC) reference strains of *Listeria* were used to evaluate the specificity of the PCR reaction. As summarized in Table 1, amplification of *Listeria grayi, L. innocua, Listeria ivanovii, Listeria seeligeri,* and *L. welshimeri* yielded single 938-bp products. When *L. monocytogenes* ATCC reference strains were used as the template, in addition to the

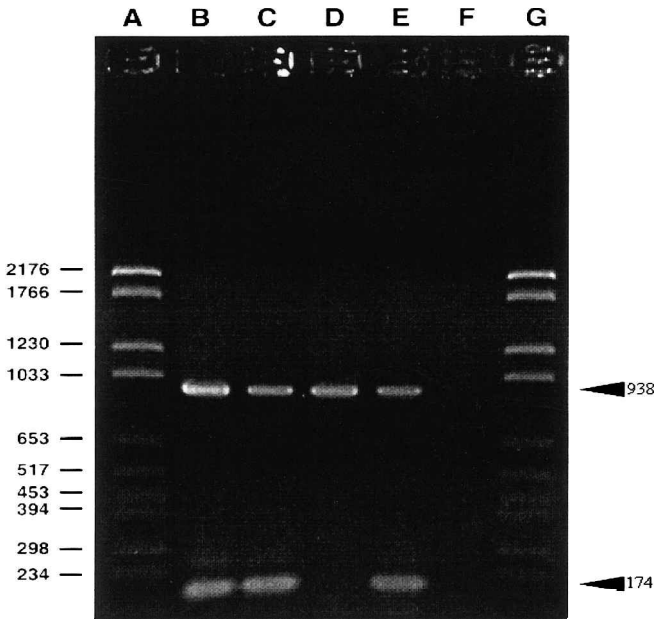

FIGURE 1. *Agarose gel electrophoresis of PCR-amplified DNAs from different* Listeria *reference strains. Lanes A and G, molecular weight marker VI; lane B, positive control (genomic DNA from* L. monocytogenes *ATCC 19111); lane C, strain NADC 3086; lane D,* L. innocua NADC 2888; lane E, L. monocytogenes *NADC 2847; lane F, negative control (mastermix without DNA). Note that* L. monocytogenes *generates both a 938- and a 174-bp amplicon. A single 938-bp product is visible for* Listeria *strains other than* L. monocytogenes.

938-bp fragment characteristic of all *Listeria* species, a unique 174-bp product was observed (Fig. 1). This pattern was evident regardless of whether the PCR reaction took place on cesium chloride–purified DNA or on a crude boiled cell extract. The 174- and 938-bp products were also observed when DNA from Centers for Disease Control reference strains of *L. monocytogenes* serotypes 1a, 1b, 1c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, and 4e were used as the template. No products were seen when purified DNA or bacterial colonies of *Erysipelothrix rhusiopathiae,* a closely related pathogen of livestock, served as the template. Likewise, no product was seen when template DNA was prepared from other gram-positive or gram-negative bacteria.

L. monocytogenes cultures recovered from three major epidemics were next evaluated to determine the specificity of the PCR reaction. Strains associated with the Los Angeles Jalisco cheese outbreak ($n = 18$), including patient and cheese isolates, yielded the 174- and 938-bp products indicative of *L. monocytogenes.* Clinical isolates recovered in the Canadian coleslaw outbreak ($n = 21$) and the single meat isolate from the 1998 multistate outbreak likewise exhibited both the 174- and the 938-bp amplicons of *L. monocytogenes.*

Additional strains of *Listeria* ($n = 55$) that had been evaluated in an earlier comparison of nucleic acid hybridization and biochemical tests to confirm *L. monocytogenes (24)* were examined. On the basis of the presence of the 174- and 938-bp amplicons, the multiplex PCR identified 41 isolates as *L. monocytogenes,* as previously confirmed.

TABLE 2. *Summary of recovery of* L. monocytogenes *and other* Listeria *species from mechanically separated turkey meat*

Trial	No. of samples positive for *L. monocytogenes* (%)	No. of samples positive for other *Listeria* spp. (%)	Total positive for all *Listeria* (%)
Trial 1 (n = 25)	19 (76)	0	19 (76)
Trial 2 (n = 25)	16 (64)	7 (28)	23 (92)
Trial 3 (n = 50)	12 (24)	7 (14)	19 (38)
Trial 4 (n = 50)	10 (20)	15 (30)	25 (50)
Total (n = 150)	57 (38)	29 (19)	86 (57)

TABLE 3. *Summary of* L. monocytogenes *serogroups isolated from mechanically separated turkey meat*

Trial	No. of samples polyvalent (1, 4)	No. of samples positive for serogroup 1	No. of samples positive for serogroup 4	No. of samples negative
Trial 1 (n = 19)[a]	18	14	4	0
Trial 2 (n = 16)	16	5	11	0
Trial 3 (n = 12)	11	5	6	1
Trial 4 (n = 10)[a]	9	5	4	0
Total (n = 57)	54	29	25	1

[a] One isolate not available for testing.

For the remaining 13 isolates, multiplex PCR yielded the single 938-bp product, verifying the identification of these isolates as *Listeria* species but not *L. monocytogenes,* as reported earlier *(19).*

Mechanically separated turkey meat sampling. Overall, *Listeria* spp. were detected in 57% (86 of 150) of the samples. *L. monocytogenes* was isolated from 38% (57 of 150) and other *Listeria* species were isolated from 19% (29 of 150) of the mechanically separated turkey meat samples (Table 2). Specifically, for trial 1, 76% (19 of 25) of the samples harbored *L. monocytogenes*; no other *Listeria* species were detected (0 of 25). For trial 2, 64% (16 of 25) of the samples tested positive for *L. monocytogenes*; an additional 28% (7 of 25) of the samples tested positive for other *Listeria* spp. For trial 3, *L. monocytogenes* was recovered from 24% (12 of 50) of the samples; 14% (7 of 50) of the samples were positive for other *Listeria* spp. For trial 4, 20% (10 of 50) of the samples yielded *L. monocytogenes*; 30% (15 of 50) harbored other *Listeria* spp.

L. monocytogenes isolates (n = 57) were serotyped and assigned to either serogroup 1 (51%, 29 of 57 isolates) or serogroup 4 (44%, 25 of 57 isolates) (Table 3). Serotyping resulted in 1 of the 57 strains being assigned to serogroups other than 1 and 4. Two of the isolates were not available for testing.

ERIC profiles of *L. monocytogenes* type 1 strains from turkey franks (n = 15) linked to an earlier listeriosis fatality *(38)* and those of mechanically separated turkey meat samples (n = 29) were compared to determine genetic similarity. By visual inspection, ERIC profiles of 13 of the 15 turkey frank isolates were found to be identical (Fig. 2). Two strains did not produce readable profiles. ERIC profiles were also generated for *L. monocytogenes* serogroup 1 recovered from mechanically separated turkey meat (n = 29 samples). Overall, 24 of the 29 samples yielded highly similar profiles. Of the *L. monocytogenes* group 1 isolates from trial 1, 86% (12 of 14) were identical and 14% (2 of 14) were different. One isolate from trial 1 was not usable and therefore was not analyzed. For trial 2, 80% (4 of 5) isolates of *L. monocytogenes* serogroup 1 exhibited identical profiles, and 1 of the 5 isolates exhibited a distinctly different profile. For trial 3, 84% (5 of 6) of the profiles were similar and 1 of the 6 profiles was different. All *L. monocytogenes* serogroup 1 isolates from trial 4 (n = 12) exhibited identical patterns. None of the profiles from mechanically separated turkey meat (n = 29) matched the ERIC profiles exhibited by isolates recovered earlier from the turkey frankfurters (n = 15).

DISCUSSION

Rapid and specific methods to detect, identify, and characterize *L. monocytogenes,* a major human foodborne pathogen, have incorporated PCR-based strategies *(3).* The aim of this study was to develop and rigorously test a multiplex PCR assay that could be used to estimate the prevalence of *Listeria* in mechanically separated turkey meat.

The specificity of the multiplex assay was extensively tested against the 6 species of *Listeria* and the 11 serotypes of *L. monocytogenes,* as well as a battery of *L. monocytogenes* strains associated with major human foodborne epidemics that occurred in North America (n = 40) and strains (n = 53) used in an earlier study to evaluate methods *(20).*

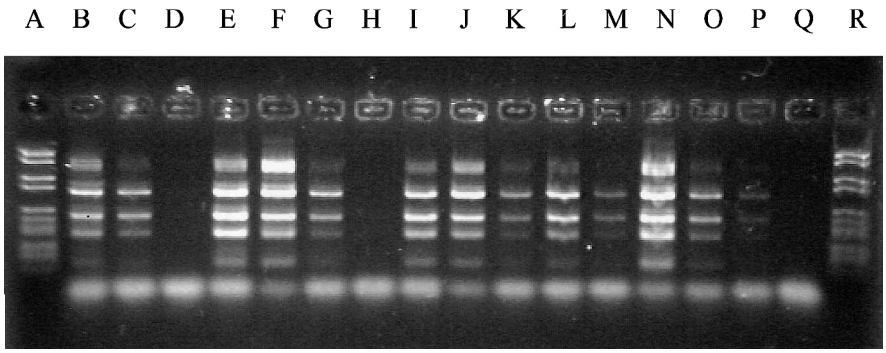

FIGURE 2. *ERIC profiles of* L. monocytogenes *serotype 1/2a recovered from turkey franks linked to a listeriosis fatality in a cancer patient. Samples in lanes E and I failed to generate patterns despite repeated attempts.*

These strains included strains recovered from the Canadian outbreak in which coleslaw was implicated ($n = 21$) and the southern California epidemic in which Mexican-style soft cheese was implicated ($n = 18$) and a single serotype 4b isolate from a meat product sampled during the most recent 1998 multistate outbreak. The resultant optimized assay is different from earlier described formats in that *L. ivanovii,* which shares a sequence homologous to that of the *hlyA* locus of *L. monocytogenes,* was not amplified. Furthermore, the assay amplifies all species of *Listeria,* including *L. grayi,* which is the most distantly related of all *Listeria* species, as well as the 11 serotypes of *L. monocytogenes.*

Carriage estimates for live chickens, based on cecal samples from parent breeders (4.7%) and 2,078 broilers (0%) *(27)* as well as broiler flock surveys (3% of 71 flocks were positive) *(28),* indicate that *L. monocytogenes* is infrequently found in live adult birds. In addition, older birds are more resistant to experimental challenge than are younger birds, the majority of which rapidly clear the infection *(40).* Studies conducted at a slaughterhouse indicate that contamination of turkey products occurs during processing, especially after evisceration and chilling *(15, 27).* In addition, the Food Safety and Inspection Service microbial baseline conducted in 1995 reported *L. monocytogenes* in carcass washes of 15% of broilers and 5.9% of turkeys. When ground products were examined, *L. monocytogenes* was cultured from 41% of ground chicken samples and 30.5% of ground turkey samples. *L. monocytogenes* has been recovered from turkey products, including 26% of 15 samples of mechanically deboned turkey meat *(10).* The difference in *L. monocytogenes* levels in live turkeys and those in turkey meat, especially ground turkey meat, suggests that a few carrier birds may cross-contaminate the final product as well as the food-processing environment. It is also probable that employees may inadvertently contribute to product contamination *(15).*

Mechanically separated turkey meat is composed of turkey skin, trim, and dark meat that is ground into a smooth paste and used later in the fabrication of delicatessen meats such as turkey frankfurters. Products derived from mechanically separated turkey meat are cooked prior to retail sale. Despite improved hygienic methods, heightened public health awareness, and increased emphasis on the hazard analysis critical control point system, the present study found that overall 57% of mechanically separated turkey meat samples were contaminated with *Listeria,* with 38% of these samples yielding *L. monocytogenes* and an additional 19% harboring other *Listeria* species. Overall contamination due to *Listeria* ranged from 38 to 92%. Factors contributing to this variation may include the high humidity in the processing plant during the summer months, when trials 1 (76%) and 2 (92%) were conducted. Comparatively lower recoveries were obtained for trials 3 and 4, which occurred during mid-September (38%) and mid-December (50%), respectively.

Serotype analysis indicated that 51% of the strains were of serogroup 1 and 44% were of serogroup 4. To estimate genetic similarity, we compared the ERIC profiles of serogroup 1 isolates recovered from mechanically separated turkey meat with those of strains associated with contaminated turkey franks linked to a listeriosis fatality *(38).* Our sole purpose was to determine if the profile exhibited by the *L. monocytogenes* serotype 1/2 strains recovered in 1988 from turkey franks currently existed in turkey products. Previously, these isolates recovered from turkey frankfurters had displayed the identical isoenzyme type *(38).* Likewise, ERIC analysis conducted in this study indicated a single pattern among the turkey frank isolates. In contrast, *L. monocytogenes* isolates of serogroup 1 collected over a 6-month interval from mechanically separated turkey meat ($n = 29$) exhibited ERIC profiles that differed from the single pattern exhibited by *L. monocytogenes* recovered from the turkey frankfurters. This finding may suggest that the strains recovered from the 1988 fatality were unique.

In conclusion, we optimized and applied a multiplex PCR for the identification of *Listeria* spp., including *L. monocytogenes.* The assay is being used in our current study to detect these species in live hogs and in pork. We have also used this multiplex PCR assay in preliminary studies to estimate the prevalence of *L. monocytogenes* (15%) and other *Listeria* species (24%) in turkey carcass rinses ($n = 80$). In this preliminary study, 10 of the 11 *L. monocytogenes* isolates were found to belong to serogroup 1, 1 was found to belong to serogroup 4, and 1 was untypable. The results of this study also indicate that serotyping in combination with DNA profiles is a relatively simple method to initially characterize this significant foodborne agent.

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